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The Levanase Operon of *Bacillus subtilis* Expressed in *Escherichia coli* Can Substitute for the Mannose Permease in Mannose Uptake and Bacteriophage Lambda Infection

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Bacteriophage lambda adsorbs to its *Escherichia coli* K-12 host by interacting with LamB, a maltose- and maltodextrin-specific porin of the outer membrane. LamB also serves as a receptor for several other bacteriophages. Lambda DNA requires, in addition to LamB, the presence of two bacterial cytoplasmic integral membrane proteins for penetration, namely, the IIC^{Man} and IID^{Man} proteins of the *E. coli* mannose transporter, a member of the sugar-specific phosphoenolpyruvate:sugar phosphotransferase system (PTS). The PTS transporters for mannose of *E. coli*, for fructose of *Bacillus subtilis*, and for sorbose of *Klebsiella pneumoniae* were shown to be highly similar to each other but significantly different from other PTS transporters. These three enzyme II complexes are the only ones to possess distinct IIC and IID transmembrane proteins. In the present work, we show that the fructose-specific permease encoded by the levanase operon of *B. subtilis* is inducible by mannose and allows mannose uptake in *B. subtilis* as well as in *E. coli*. Moreover, we show that the *B. subtilis* permease can substitute for the *E. coli* mannose permease cytoplasmic membrane components for phage lambda infection. In contrast, a series of other bacteriophages, also using the LamB protein as a cell surface receptor, do not require the mannose transporter for infection.

Bacteriophages recognize their host bacteria through highly specific binding to receptors located at the bacterial cell surface (for a review, see reference 14). Bacteriophage λ adsorbs to its *Escherichia coli* K-12 host by interacting with the outer membrane protein LamB. This protein was thus initially named the λ receptor (24, 39). LamB also serves as a specific cell surface receptor for several other bacteriophages (4). It is a trimeric protein that forms nonspecific channels through the outer membrane, allowing the diffusion of small hydrophilic molecules (<600 Da). In addition, it is a maltose- and maltodextrin-specific porin that facilitates the diffusion of maltose and maltodextrins into the cell. The three-dimensional structure of LamB was recently determined by X-ray crystallography (36).

The mechanisms by which λ injects its DNA into the cell are still poorly understood. Previous in vivo and in vitro studies (2, 37) have shown that the adsorption of λ to the LamB protein occurs into two major steps: a reversible interaction followed by an irreversible interaction which appears to be a prerequisite for DNA ejection. Bacteriophage λ requires, in addition to LamB, the presence of two cytoplasmic membrane proteins for DNA penetration (11, 12, 34, 35). These two integral membrane proteins, IIC^{Man} and IID^{Man}, are constituents of the mannose transporter in *E. coli*, a member of the sugar-specific phosphoenolpyruvate:sugar phosphotransferase system (PTS), which is responsible for the uptake and concomitant phosphorylation of a number of sugars in both gram-negative and gram-positive bacteria (for reviews, see references 23 and 33).

The sugar-specific PTS proteins which comprise the enzyme II complexes consist of three to four proteins or protein domains termed IIA, IIB, IIC, and IID (23, 32, 33). IIA and IIB are phosphoryl-transfer proteins or protein domains that are either cytoplasmic or localized to the cytoplasmic side of the bacterial membrane. The IIC and IID proteins or proteins domains are integral membrane constituents. The mannose transporter of the PTS in *E. coli* has a broad substrate specificity for mannose, glucose, related hexoses, and fructose. The PTS transporters for mannose of *E. coli* (12), for fructose of *B. subtilis* (19), and for sorbose of *Klebsiella pneumoniae* (41) are homologous to each other but very different from other PTS transporters. These three enzyme II complexes comprise the so-called splinter group of PTS permeases that differ in structure and phylogenic origin from other sequenced PTS permeases since they all possess two distinct IIC and IID transmembrane proteins (26). Moreover, it was recently shown that the *K. pneumoniae* sorbose-inducible sorbose transporter could serve as a transport system for fructose and, conversely, that the *E. coli* soluble bidomain protein $IIAB^{Man}$ could complement the lack of IIA^{Sor} and IIB^{Sor} soluble components (41), confirming the functional relatedness of these two highly homologous systems.

Systematic sequencing of the *E. coli* genome has revealed a number of previously unrecognized genes encoding putative proteins of the PTS (25–27). Genes encoding PTS proteins are scattered randomly on the bacterial chromosome and represent over 1% of all currently sequenced *E. coli* genes. Very recently, the analysis of the 67.4- to 76.0-min region of *E. coli* revealed the presence of five genes encoding homologs of the *E. coli* mannose permease (IIB, IIB′, IIC, IIC′, and IID proteins) (26), which may be involved in an *N*-acetylgalactosamine-6-phosphate metabolic pathway. In particular, the protein products of the *agaC* gene (named IIC^{Aga}) and the *agaD* gene (named IID^{Aga}) are full-length proteins similar to the *E. coli* mannose, *K. pneumoniae* sorbose, and *B. subtilis* fructose IIC and IID integral membrane proteins, respectively.

In *B. subtilis*, the levanase operon, which is inducible by

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TABLE 1. Bacteria and phages used in this study

Bacterial strain or phage	Relevant genotype	Source and/or reference(s)
E. coli		
921	F$^-$ *thr leu met lac supE*$^+$ (r_K^- m_K^-)	Pel$^+$ (34)
WA2127	Spontaneous λ-resistant mutant of 921; *manXYZ*	Pel$^-$ (IIAB^{Man-} IIC^{Man-} IID^{Man-}) (34)
WA3155	Spontaneous mutant of 921; *manY*	Pel$^-$ (IIC^{Man-}) (11, 12, 34)
ZSC113	F$^-$ *gpt-2 mpt-2 glk-7 strA manZ ptsG*	(IID^{Man-}) (6)
WA2127/pHT315	WA2127 transformed with pHT315	This work
WA2127/pRL3	WA2127 transformed with pRL3	This work
WA3155/pHT315	WA3155 transformed with pHT315	This work
WA3155/pRL3	WA3155 transformed with pRL3	This work
ZSC113/pHT315	ZSC113 transformed with pHT315	This work
ZSC113/pRL3	ZSC113 transformed with pRL3	This work
B. subtilis		
168	*trpC2*	Laboratory collection
QB 5050	*trpC2 levR8 sacC-lacZ*	19
QB 5054	*trpC2 levR8 levG-lacZ*	19
QB 5081	*trpC2 levD-lacZ*	20
Phages		
λvh$^+$	λ virulent phage with a wild-type host range	λb2 *vir h*$^+$ (3)
λvh°	One-step extended host range mutant of λh$^+$	λb2 *vir h*° (3)
λvhh*	Two-step extended host range mutant of λh$^+$	λb2 *vir hh** (3)
λ*c*I57h$^+$	λ clear-plating phage with wild-type host range	Laboratory collection
λ*c*I57hpel-1	Mutant of λ*c*I57h$^+$ selected on the Pel$^-$ strain WA2127	This work
λ*c*I57hpel-2	Mutant of λ*c*I57h$^+$ selected on the Pel$^-$ strain WA2127	This work
λ*c*I57hpel-3	Mutant of λ*c*I57h$^+$ selected on the Pel$^-$ strain WA2127	This work
λ*c*I57hpel-4	Mutant of λ*c*I57h$^+$ selected on the Pel$^-$ strain WA2127	This work
λ*c*I57h434	λ clear-plating recombinant phage between λ*c*I and 434 with the host range of 434	Laboratory collection
AC6	LamB-specific phage	4
AC7	LamB-specific phage	4
21EL	LamB-specific phage	4
AC28	LamB-specific phage	4
AC30	LamB-specific phage	4
AC50	LamB-specific phage	4
AC57	LamB-specific phage	4
AC81	LamB-specific phage	4
AC95	LamB-specific phage	4
K10	LamB-specific phage	29
Tu1a	OmpF-specific phage	7
TuII*	OmpA-specific phage	7
TP1	Uses either LamB or OmpF as a receptor	40
AB48	Uses either LamB or OmpC as a receptor	4

fructose, comprises five genes (19). The first four genes (*levD*, *levE*, *levF*, and *levG*) encode polypeptides that are similar to the proteins of the mannose-PTS family. The *lev*-PTS is a minor fructose-PTS in *B. subtilis* which is involved in the regulation of transcription of the levanase operon (19). LevD, LevE, LevF, and LevG correspond to IIA^{Fru}, IIB^{Fru}, IIC^{Fru}, and IID^{Fru}, respectively, according to the PTS uniform nomenclature (32). The fifth gene, *sacC*, encodes levanase, an enzyme that can hydrolyze sucrose and degrade fructose polymers. The levanase operon is transcribed from a (−12, −24) promoter recognized by a specific sigma factor, σ^L, which is equivalent to σ^{54} in gram-negative bacteria (9). The expression of the operon is positively regulated by the LevR activator, a member of the NiFA/NtrC family of regulators (8).

We report here that the levanase operon of *B. subtilis* is also inducible by mannose and promotes mannose uptake in *B. subtilis* as well as in *E. coli*. In addition, we found that the *B. subtilis* cytoplasmic components IIC^{Fru} and IID^{Fru} could substitute for their *E. coli* mannose homologs to allow phage λ DNA injection. The functional implications of these observations are discussed in terms of the mechanism of phage λ infection.

MATERIALS AND METHODS

Strains, media, and chemicals. Phages and bacterial strains are listed in Table 1. The parental *E. coli* K-12 strain 921 and its derivatives WA2127 (*manXYZ* mutant strain, IIAB^{Man-} IIC^{Man-} IID^{Man-}) and WA3155 (*manY* mutant strain, IIC^{Man-}) were previously described (12, 34). The *manZ* mutant strain ZSC113 (IID^{Man-}) was described in reference 6. The relevant genotypes of the strains were deduced from the results of complementation assays with recombinant plasmids (12) or from genetic mapping (6). The term "Pel" (for penetration of lambda), used for simplification in the text, refers to the historical nomenclature (34). A Pel$^+$ phenotype corresponds to wild-type mannose operon-encoded proteins, while Pel$^-$ corresponds to mutations in the IIC^{Man} and/or IID^{Man} protein.

Phages that in this paper are called λ*c*I57hpel-1 to λ*c*I57hpel-4, which were

TABLE 2. EOP of the phages on the different *E. coli* strains

Strain	EOP of phage[a] in:							
	Category I				Category II			Category III (λ*c*I57h434)
	I (λvh$^+$)	I (λvh°)	I (λvhh*)	I′ (λ*c*I57h$^+$)	II (AC phages)[b]	II′ (K10)	II″ (λ*c*I57hpel)[b]	
921 (Pel+)	1	1	1	1	1	1	1	1
WA2127 (IIC^{Man-} IID^{Man-})	1×10^{-8}	1×10^{-8}	1×10^{-8}	2.5×10^{-6}	1	1	0.1	1.5×10^{-6}
WA3155 (IIC^{Man-})	1×10^{-8}	1×10^{-8}	1×10^{-8}	2×10^{-6}	1	1	0.15	5×10^{-5}
ZSC113 (IID^{Man-})	1	1	1	5×10^{-3}	1	2×10^{-4}	5×10^{-3}	0.2
WA2127/pHT315	1×10^{-8}	1×10^{-8}	1×10^{-8}	3×10^{-6}	1	1	0.2	2.5×10^{-6}
WA2127/pRL3	0.5	0.5	0.5	0.5	1	1	1	0.25
WA3155/pHT315	1×10^{-8}	1×10^{-8}	1×10^{-8}	2×10^{-6}	1	1	0.15	5×10^{-6}
WA3155/pRL3	1	1	1	0.5	1	1	0.15	0.12
ZSC113/pHT315	1	1	1	5×10^{-3}	1	2×10^{-4}	5×10^{-3}	0.5
ZSC113/pRL3	1	1	1	5×10^{-2}	1	2×10^{-4}	5×10^{-2}	0.1

[a] The phages were grouped into three main categories according to their ability to plate on the different *E. coli* mannose-negative mutants WA2127, WA3155, and ZSC113. Within each category, the prime and double prime illustrate a slight difference in the EOP compared to that of the other members of the category.

[b] The nine AC phages listed in Table 1 (AC6, AC7, AC28, AC30, AC43, AC50, AC57, AC81, and AC95) and 21EL were tested by a spot test (10 μl of 10^{-2}, 10^{-4}, and 10^{-6} dilutions spotted on the same lawn of the mutant, on tryptone rich solid medium). All the phages showed approximately the same EOP on the different strains. Therefore, precise EOP values were determined for only four of them: AC6, AC7, AC28, AC30, AC43, and 21EL (as described in Materials and Methods).

obtained in this study, are four spontaneous mutants of phage λ*c*I57h$^+$ (15) isolated on the Pel$^-$ strain WA2127. Mutant phages able to plate on WA2127 were obtained at a frequency of approximately 10^{-6} (see Table 2).

The *B. subtilis-E. coli* shuttle plasmid pRL3, carrying the *levR8* allele and the first four genes of the levanase operon encoding the *lev* PTS and plasmid pHT315 (negative control without insert), were described previously (8). They were introduced into the different *E. coli* mutants by electroporation, followed by selection on Luria-Bertani (LB) medium containing ampicillin (final concentration, 100 μg/ml), as previously described (16). The plasmids in the transformed cells were checked by restriction enzyme analysis.

E. coli was grown in M63 minimal medium. The ability of the recombinant strains to utilize mannose as a carbon source was assayed by streaking on MacConkey mannose (1% final) solid medium. Bacteria that are able to use mannose form red (Man$^+$ phenotype) or pink (Man$^{+/-}$ phenotype) colonies on this medium, while bacteria that are unable to use mannose form white colonies (Man$^-$ phenotype). The media and chemicals were as described in reference 5.

Phage sensitivity assays. The sensitivity of the *E. coli* recombinant bacteria to a series of bacteriophages was tested as previously described (5). Briefly, 100 μl of each phage dilution (in M63 liquid medium) was incubated for 15 min at room temperature with 100 μl of bacterial culture in LB medium (plus ampicillin if required) before being spread onto LB plates by the tryptone overlay method. We tested 10^{-2}- to 10^{-8}-fold dilutions of the phage stocks. The efficiencies of plating (EOP) of the phages on the different bacterial strains are listed in Table 2. They correspond to the ratio between the titer of the phage stock recorded on the mutant and the titer recorded on the wild-type Pel$^+$ strain 921 (hence, an EOP of 1 indicates that the phage has the same titer on the mutant as on the wild-type strain). The EOP reflects the ability of the phage to infect productively the bacterial host, i.e., its ability to adsorb to the cell surface receptor, LamB, to inject its DNA through the bacterial envelope, to undergo its lytic cycle in the cytoplasm of the infected cell, and finally to release the progeny phage.

β-Galactosidase assays. *B. subtilis* was grown in C medium [70 mM $K_2HPO_4 \cdot 3H_2O$, 30 mM KH_2PO_4, 25 mM $(NH_4)_2SO_4$, 0.5 mM $MgSO_4$, 10 μM $MnSO_4$, 22 mg of ferric ammonium citrate per liter, 100 mg of auxotrophic requirements per liter). CSK medium is C medium supplemented with potassium succinate (6 g/liter) and potassium glutamate (8 g/liter). β-Galactosidase activities were measured, after the growth of *B. subtilis* strains in CSK medium supplemented with increasing concentrations of mannose (0 to 27 mM; see Fig. 1C), by the method of Miller (21). One unit of β-galactosidase activity is defined as the amount of enzyme which produces 1 nmol of *o*-nitrophenol per min at 28°C.

In vivo mannose uptake assays in *B. subtilis* and in *E. coli* K-12. *B. subtilis* strains were grown in CSK medium, and *E. coli* was grown in M63 supplemented with 4 g of glycerol per liter, 0.025 g of casein hydrolysate per liter, and auxotrophic requirements at 100 mg per liter. Sugar uptake assays were performed as described previously (10). Medium C or M63 supplemented with 10 g of glycerol per liter and 250 mg of chloramphenicol per liter was used for incorporation. Exponentially growing cells were harvested by centrifugation at an optical density at 600 nm of 0.6 to 0.8 and washed once with the incorporation medium. The cells were incubated at 37°C with shaking. Labeled [^{14}C]mannose (30 kBq per ml) and nonlabeled mannose (final concentration, 0.3 mM) were added. Samples (0.5 ml) were withdrawn at intervals and filtered through Millipore membranes (pore size, 0.45 μm). The filters were washed with 15 ml of the incorporation medium at 37°C and dried, and radioactivity was measured by scintillation counting.

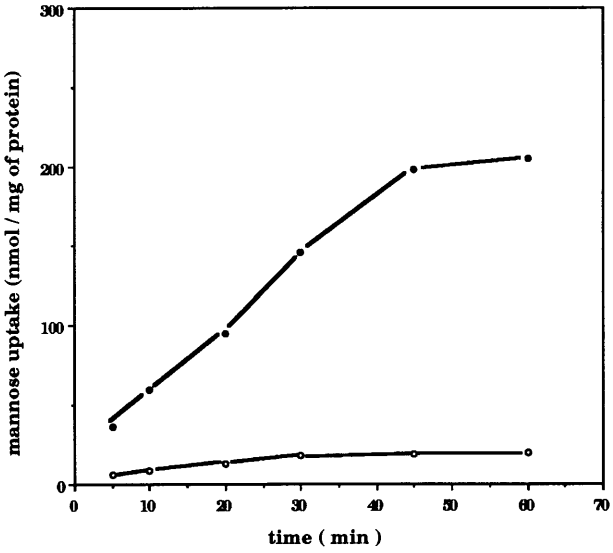
RESULTS

We first studied the participation of the fructose-PTS encoded by the levanase operon of *B. subtilis* in mannose transport in *B. subtilis* as well as in *E. coli*. In addition, we investigated if the *lev*-PTS of *B. subtilis* could replace the *E. coli* mannose permease in its participation in the infection of *E. coli* K-12 by λ and other phages. These two distinct aspects are presented below.

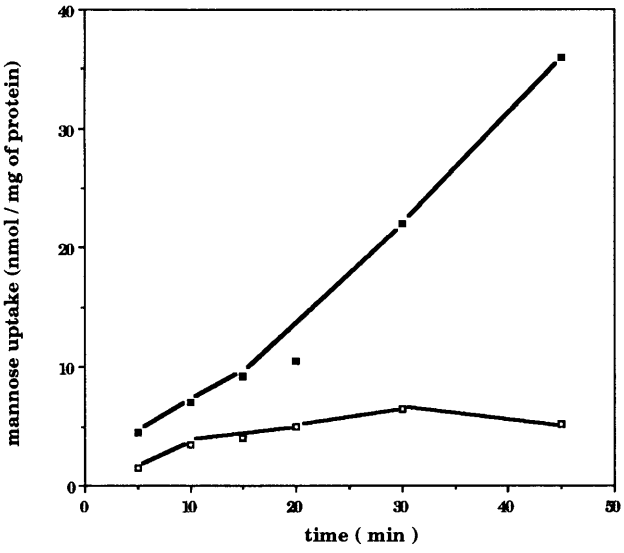
The levanase operon of *B. subtilis* is inducible by mannose and promotes mannose uptake in *B. subtilis* as well as in *E. coli*. (i) Mannose uptake in *B. subtilis*. Little is known about mannose utilization in *B. subtilis*. Mannose is probably taken up via a PTS since a *ptsI* mutant of *B. subtilis* does not grow on mannose (13). The *lev*-PTS of *B. subtilis* is similar in sequence to the mannose-PTS of *E. coli* (see Discussion). We therefore tested the participation of the levanase operon in mannose uptake. We first measured in vivo mannose uptake in the wild-type strain *B. subtilis* 168 after growth in the presence or absence of 27 mM mannose (Fig. 1A). An inducible mannose uptake (4.75 nmol min^{-1} mg^{-1}) was observed in this strain. This uptake was not abolished when the *levG* gene was disrupted (data not shown), indicating that another major inducible mannose transporter different from the *lev*-PTS exists in *B. subtilis*.

To distinguish mannose transport due to LevD, LevE, LevF, and LevG from that of other systems, *levR8* mutants were used. In these mutants, the levanase operon is expressed constitutively but the other mannose-PTS are not, since they require induction by mannose (Fig. 1A). Strains QB5050 (*levR8*) and QB5054 (*levR8 levG-lacZ*) were grown in CSK medium, and [^{14}C]mannose uptake was assayed (Fig. 1B). Constitutive mannose uptake was observed in strain QB5050 (*levR8*), and this uptake was abolished when a *lacZ* cassette (Table 1) was introduced into *levG*, confirming that mannose was indeed taken up by the *lev*-PTS.

(ii) Induction of the levanase operon of *B. subtilis* in the presence of increasing concentrations of mannose. We previously showed that the levanase operon was specifically induced



A



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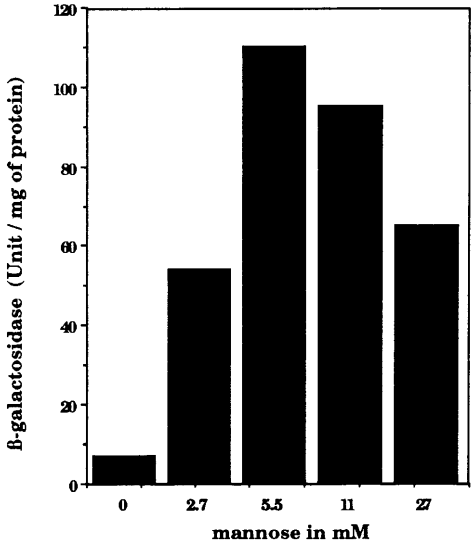

C

FIG. 1. Mannose utilization in *B. subtilis*. (A) Inducible mannose uptake in wild-type *B. subtilis* 168. Measurements of [^{14}C]mannose uptake were performed as described in Materials and Methods after growth of strain 168 in CSK (○) or in CSK in the presence of 27 mM mannose (●). (B) Constitutive mannose uptake via the *lev*-PTS. Constitutive [^{14}C]mannose uptake was measured after growth in CSK of strain QB5050 (*levR8*) (■) and strain QB5054 (*levR8 levG-lacZ*) (□). (C) Effect of increasing concentrations of mannose on the expression of a *levD-lacZ* translational fusion. Liquid cultures of strain QB5081 were grown at 37°C in CSK medium in the presence of different mannose concentrations (0 to 27 mM). β-Galactosidase specific activities were determined in extracts prepared from exponentially growing cells (optical density at 600 nm, 0.6 to 0.8 for CSK and 0.8 to 1 for CSK with mannose).

by fructose in *B. subtilis* (18). This induction is mediated by the *lev*-PTS, probably by phosphorylating the activator protein LevR in response to the availability of fructose (8, 19). Since the *lev*-PTS was able to take up mannose in addition to fructose, we hypothesized that expression of the levanase operon could also be inducible by mannose. The expression of a *levD-lacZ* translational fusion (named PΔB [20]) was measured after growth in CSK medium in the presence of increasing concentrations of mannose (0 to 27 mM) (Fig. 1C). The rate of synthesis of β-galactosidase was dependent on the mannose concentration, with an optimum at 5.5 mM, where a 15-fold induction by mannose was recorded. Expression of the operon in the presence of increasing concentrations of fructose led to a bell-shaped curve as observed for mannose. This inhibitory effect at high concentration is probably due to carbon catabolite repression (18).

(iii) The *B. subtilis* fructose permease allows mannose uptake in *E. coli* K-12 mannose-negative mutants. Confirming previous observations, the three *E. coli man*-negative mutants WA2127 (IIAB^{Man-} IIC^{Man-} IID^{Man-}), WA3155 (IIC^{Man-}), and ZSC113 (IID^{Man-}) were unable to ferment mannose on MacConkey mannose solid medium (Table 3). Plasmid pRL3 was introduced into the three *E. coli* mutants (see Materials and Methods). This plasmid carries the *levD*, *levE*, *levF*, and *levG* genes under the control of the (−12, −24) promoter of the levanase operon and the *levR8* allele, which encodes a constitutive LevR activator (8). The three types of transformants regained the capacity to ferment mannose, indicating that the *lev*-PTS proteins were expressed in *E. coli* and could complement the lack of either one (IIC^{Man} in mutant WA3155 and IID^{Man} in mutant ZSC113) or three (IIC^{Man}, IID^{Man}, and IIAB^{Man} in mutant WA2127) components of the *E. coli* mannose transporter. In this test, mutant ZSC113/pRL3 appeared to be the best-fermenting strain.

To confirm these observations, we measured in vivo mannose uptake in the *E. coli* recombinants (as described in Materials and Methods). Strains ZSC113, WA3155, and WA2127, transformed with plasmid pHT315 (1), were also tested as negative controls. The rates of mannose uptake in strains ZSC113/pHT315, WA3155/pHT315, and WA2127/pHT315 were 0.4, 0.5, and 0.45 nmol of mannose min^{-1} mg of protein^{-1}, respectively (Table 3). Introduction of plasmid pRL3 led to a 9- to 14-fold increase in mannose transport, with a rate

TABLE 3. Mannose utilization in *E. coli* K-12

E. coli strain	Plasmid	Phenotype[a]	Mannose uptake (nmol/min/mg of protein)[b]
WA2127	pHT315	−	0.45
	pRL3	+	4.5
WA3155	pHT315	−	0.5
	pRL3	+	4.4
ZSC113	pHT315	−	0.4
	pRL3	++	5.6

[a] The phenotypes of the different strains containing plasmids pRL3 or pHT315 were tested after growth on MacConkey mannose solid medium (final concentration of mannose, 1%). Symbols: −, white colonies; +, pink to red colonies; ++, dark red colonies.

[b] In vivo mannose uptake in the *E. coli* recombinants was assayed as described in Materials and Methods.

of uptake of 5.6 nmol min^{-1} mg of $protein^{-1}$ for strain ZSC113/pRL3, 4.4 nmol min^{-1} mg of $protein^{-1}$ for WA3155/pRL3, and 4.5 nmol min^{-1} mg of $protein^{-1}$ for WA2127/pRL3. Thus, the *lev*-PTS is able to restore mannose uptake in *E. coli* mutants inactivated for the mannose transporter.

The proteins encoded by the *B. subtilis* levanase operon restore phage sensitivity to *E. coli* Pel⁻ mutants. It was previously shown that phage λ requires, in addition to the outer membrane protein LamB, inner membrane components involved in mannose uptake to infect *E. coli* K-12 (11, 34, 35). More recently, Erni et al. (12) showed that only the IIC^{Man} and IID^{Man} cytoplasmic membrane components of the mannose transporter were involved in phage λ DNA injection.

We have tested the ability of the *B. subtilis* permease to compensate for the lack of either IIC^{Man} and/or IID^{Man} components in the infection of *E. coli* K-12 by phage λ and by other phages known to use the outer membrane protein LamB as a cell surface receptor. We used the three *E. coli* mannose permease mutants WA2127 ($IIAB^{Man-}$ IIC^{Man-} IID^{Man-}), WA3155 (IIC^{Man-}), and ZSC113 (IID^{Man-}) transformed with either plasmid pRL3 (*B. subtilis lev*-PTS genes) or pHT315 (negative control). The sensitivity to phages was determined by measuring the EOP of the phages on the different bacterial strains (see Materials and Methods).

We first tested the sensitivity of the different bacterial strains to two mutants of phage λ with a wild-type host range (i.e., able to recognize the wild-type LamB receptor but not LamB mutants resistant to λh^+): the virulent phage λb2virh$^+$ (2), noted to be λvh^+ (and carrying the *b2* deletion), and phage λ*c*I57h$^+$ (a clear-plating mutant of λ with a mutation in gene *c*I). The sensitivity to the one-step and two-step extended-host-range derivatives of λvh^+ (λvh° and λvhh*, respectively) was also determined. Then we tested the recombinant bacteria for sensitivity to a series of previously isolated λ-like phages (4) by using LamB as a specific cell surface receptor (these phages named AC followed by a number [Table 1]), as well as to K10 (29), another LamB-specific phage. All these phages make clear plaques on their *E. coli* host. Finally, we used phage λ*c*I57h$^+$ to select four independent spontaneous mutant phages that were able to plate on the Pel$^-$ strain WA2127. Stocks from these four mutant phages were then prepared on the Pel$^+$ strain 921 and tested on the different recombinant bacteria (Table 2).

In addition, we tested several phages by using outer membrane proteins other than LamB as cell surface receptors; these phages included (i) λ*c*I57h434, a recombinant between λ (λ*c*I57) and phage 434 with the immunity of λ and the host range of 434, using the outer membrane protein OmpC as a receptor (2); (ii) phage Tu 1a (7), using OmpF as a receptor; (iii) phage Tu II*, using OmpA as a receptor; and (iv) two phages that are alternate users of either LamB or OmpF (TP1) (40) and either LamB or OmpC (AB48) (4).

The phages could be grouped into three main categories in terms of their EOP on the different bacterial strains tested (Table 2).

Category I includes λvh^+, its one-step and two-step extended host range derivatives (λvh° and λvhh*), and λ*c*I57h$^+$. The EOP of these four phages was strongly reduced in the IIC/IID^{Man-} mutant (WA2127) and in the single IIC^{Man-} mutant (WA3155): approximately 10^{-8} for λvh^+, λvh°, and λvhh* and 10^{-6} for λ*c*I57h$^+$. As previously reported, the ability of phage λ to grow on Pel$^-$ hosts is influenced by the DNA content of the λ phage particles (11). In particular, deletion mutants with mutations in the replaceable *b* region of the λ genome were shown to have reduced EOP on Pel$^-$ bacteria compared to that of wild-type λ. This observation is in agreement with the lower EOP of λvh^+ and extended-host-range derivatives (which carry the *b2* deletion) compared to that of λ*c*I57h$^+$.

In contrast, the mutation in the IID^{Man} subunit (strain ZSC113) had no effect on the EOP of λvh^+, λvh°, and λvhh* and only a moderate effect on that of λ*c*I57h$^+$ (10^{-2} to 10^{-3}).

Introduction of plasmid pRL3 into the two Pel$^-$ strains WA2127 and WA3155 restored wild-type sensitivity to these four phages (EOP of 0.5 to 1 [Table 2]). This result demonstrates that the *B. subtilis* permease expressed in *E. coli* could substitute for the mannose permease IIC and IID subunits in their participation in the infection by lambda.

The EOP of λ*c*I57h$^+$ on the IID^{Man-} mutant appeared to be reduced (5×10^{-3} instead of 1 for the three λvir phages), and it was only slightly improved by the introduction of pRL3 into the strain (5×10^{-2}), suggesting that IID^{Fru} did not completely restore phage injection. λ*c*I57h$^+$ was therefore distinguished from the three other phages of the category (category I′, Table 2).

Category II includes all nine AC phages (Table 1), K10, and the four hpel derivatives of λ*c*I57h$^+$ (λ*c*I57hpel-1 to λ*c*I57hpel-4). Strikingly, the nine AC phages and K10 plated on the Pel$^-$ mutant WA2127 as well as on the parental Pel$^+$ strain 921 (EOP of 1), demonstrating that these phages, in contrast to λ, do not require the mannose permease to infect their *E. coli* host. The nine AC phages plated equally well on the three mannose permease mutants. In contrast, the EOP of K10 recorded on the IID^{Man-} mutant ZSC113 was reduced (2×10^{-4}) and was not improved by the introduction of plasmid pRL3. Therefore, K10 was classified as a category II′ phage (Table 2).

In agreement with previous observations (12), the four λ*c*I57hpel mutants plated slightly better on the parental Pel$^+$ strain than on the Pel$^-$ mutant WA2127, on which they had been isolated (1 log unit difference). Introduction of pRL3 into WA2127 restored wild-type sensitivity to these phages. Introduction of pRL3 in WA3155 did not improve the EOP of the λ*c*I57hpel mutant tested (mutant 3); and in ZSC113 there was only a slight improvement (1 log unit [Table 2]).

Category III contains only λ*c*I57h434. This phage is composed of the right portion of λ (comprizing the early transcribed genes including *c*I) and the left portion of 434 (which carries the late transcribed genes including head and tail genes). It utilizes the outer membrane protein OmpC as a receptor.

Like λh^+, λ*c*I57h434 was also dependent on the presence of the mannose transporter for infection, as shown by its reduced EOP on WA2127 (10^{-6}). The lack of the IIC^{Man} subunit alone also significantly reduced the ability of the phage to infect *E.*

coli (EOP of 10^{-5}). Strikingly, the mutation in the IID^{Man} subunit in ZSC113 had only very little, if any, effect on infection by this phage.

We finally tested as controls (not included in Table 2) the sensitivity of the Pel^+ strain 921 and the Pel^- strain WA2127 to four other non-λ-like phages: phage Tu1a (using OmpF as the surface receptor), phage TuII* (using OmpA) (7), and two phages, TP1 and AB48 (using either LamB or OmpF and either LamB or OmpC, respectively) (4, 40). The EOP of the four phages was identical on the two *E. coli* strains, indicating that these phages do not require the PTS proteins for infection.

DISCUSSION

We showed in this work that the levanase operon of *B. subtilis* encoding a fructose-inducible fructose-specific PTS was also inducible by mannose and promoted mannose uptake in *B. subtilis* in addition to fructose uptake. We also showed that the *lev*-PTS, expressed in *E. coli*, could function in mannose uptake. Moreover, we found that the *B. subtilis* membrane proteins IIC^{Fru} and IID^{Fru} could substitute for their *E. coli* homologs IIC^{Man} and IID^{Man} to allow infection of *E. coli* by bacteriophage λ. Finally, we report that other λ-like phages, using the LamB protein as a specific cell surface receptor, do not require the mannose permease for infection.

The proteins determined by the levanase operon of *B. subtilis* can mediate mannose uptake in *B. subtilis* and in *E. coli*. We first tested the inducibility of the levanase operon genes by mannose in *B. subtilis*, by using a *levD-lacZ* translational fusion. At the optimal mannose concentration of 5.5 mM (Fig. 1C), a 15-fold induction of the protein fusion was recorded, demonstrating that the levanase operon expression was inducible both by fructose and by mannose.

We then tested the ability of the levanase operon to catalyze mannose uptake. We observed that in vivo mannose uptake was inducible by mannose in the wild-type *B. subtilis* 168 (Fig. 1A). To establish that the *lev*-PTS could also take up mannose, mutants expressing the levanase operon constitutively (*levR8* mutants) were used (8). In the *levR8* mutant expressing the wild-type levanase operon, constitutive in vivo mannose uptake was observed, while in its derivative carrying an inactivated *levG* gene, this uptake was abolished (Fig. 1B). These data demonstrate that the *lev*-PTS of *B. subtilis* is able to take up mannose, in addition to the previously shown fructose uptake (19). Moreover, mannose transport via the *lev*-PTS and inducibility of the levanase operon by this sugar reinforce the hypothesis of a link between the uptake via the *lev*-PTS and the modulation of transcription of the levanase operon.

Finally, the ability of the levanase operon to restore mannose uptake in three different *E. coli* mannose-negative mutants was tested. Introduction of plasmid pRL3 carrying the constitutively expressed *lev*-PTS genes restored efficient in vivo mannose uptake in each case, with a 9- to 14-fold increase of the basal activity (Table 3). This result demonstrates that the *lev*-PTS of *B. subtilis* is able to substitute for the *E. coli* mannose transporter for mannose uptake in *E. coli.*

As described previously, the proteins of the fructose transporter of *B. subtilis* are homologous to the proteins of the mannose transporter of *E. coli* (17, 28). Moreover, it was recently shown that the *K. pneumoniae* sorbose-inducible sorbose transporter could also serve as a transport system for fructose (41). These three fructose enzyme II complexes are the only PTS permeases to possess two distinct IIC and IID transmembrane proteins. The recently discovered Aga system provides another example (26). It is tempting to propose that this unique structural organization is responsible for their extended substrate specificity.

The fructose permease of *B. subtilis* can complement the lack of the *E. coli* IIC^{Man} and IID^{Man} proteins for phage λ infection. Previous genetic analyses of LamB mutants blocking phage λ adsorption allowed the identification of the "phage λ receptor site" at the surface of the LamB protein (3, 22, 36). Mutants of λ able to use such mutated LamB receptors were selected (15). These phages, which could still use the wild-type receptor, were called extended-host-range mutants of λ. We recently showed (42) that the mutations in these mutated λ phages were all located in the C-terminal 10% portion of the J protein (the tail fiber protein of λ [38]), strongly suggesting that the C terminus of J was directly involved in LamB recognition (42).

In Pel^- mutants, reversible adsorption of λ to LamB is not blocked but DNA injection is inhibited. Mutants of λ (initially called λhpel) that are able to plate on Pel^- hosts can be selected (35; this work). The λhpel mutants previously studied had alterations in one of two components of the phage tail (either in gpV, the tail tube protein, or in gpH, another component of the tail) (35), suggesting a direct interaction between the cytoplasmic membrane proteins of the mannose transporter and these phage tail proteins.

(i) Complementation for phage infection of mutations in the *E. coli* mannose permease by the *B. subtilis lev*-PTS-encoded proteins. The two Pel^- mutants WA2127 and WA3155 were resistant to the four λ derivatives tested (category I in Table 2), as shown by the drastic reduction of the EOP of these phages on the two *E. coli* mutant strains (10^{-6} to 10^{-8}). Introduction of plasmid pRL3, carrying the *lev*-PTS of *B. subtilis*, into these two strains restored wild-type sensitivity to these four phages. This result demonstrates that the *B. subtilis* permease expressed in *E. coli* can substitute for the IIC/IID^{Man} protein complex in its participation in λ infection.

Like λ, phage λ*c*I57h434 (a recombinant between phage λ*c*I57 and phage 434 [see Results]) was dependent on the presence of the mannose transporter for infection. The lack of the IIC^{Man} subunit also considerably reduced the ability of this phage to infect *E. coli*. This result indicates that λ and 434, which use different cell surface receptors, probably use the same machinery at the level of the inner membrane to inject their DNA.

The IID^{Man-} mutant (strain ZSC113) had no effect on infection by phages λ*c*I57h434, λvh$^+$, λvh°, and λvhh* and only a moderate effect on infection by λ*c*I57h$^+$. However, in this mutant, mannose uptake was completely abolished (see above). The same types of defects were also reported for C-terminal truncations of either IIC^{Man} or IID^{Man} (12). Both types of deletions were shown to abolish mannose transport but affected phage sensitivity only moderately and to various extents. In the absence of precise topological information on the IIC/IID^{Man} complex, one may suggest that the C termini of IIC^{Man} and IID^{Man} are important only for proper interaction with the $IIAB^{Man}$ cytoplasmic phosphoryl-transfer protein, which is required for sugar transport and concomitant phosphorylation.

Strikingly, none of the 10 other phages that also use LamB as a specific cell surface receptor (4, 29) required the presence of the mannose permease for infection (category II in Table 2). These data indicate clearly that the machinery used by these 10 LamB-specific phages to infect *E. coli* is distinct from that used by λ for infection.

(iii) Implications for the mechanism of phage infection. Phage adsorption, the initial step of phage infection, has been studied for many different phages and is well documented (for

a review, see reference 14). In contrast, the mechanisms of penetration of phage DNA into bacterial cells remain poorly understood. The available in vitro and in vivo data on λ infection (references 3, 30, 31, and 42 and references therein) suggest that reversible λ adsorption involves only the recognition and binding of the C-terminal portion of the J protein (at the tip of the tail fiber) to its receptor site (at the surface of the LamB protein). In contrast, the passage to irreversible binding and DNA injection corresponds to major conformational changes both in the phage tail and in the bacterial outer and inner membrane components.

The present study showed that phages using the same outer membrane protein for adsorption onto their *E. coli* host may or may not require the participation of the mannose permease for infection. In spite of important differences in their primary sequences, the IIC/IID^{Fru} protein complex of *B. subtilis* could substitute for the IIC/IID^{Man} complex in λ infection (26). The conserved residues are scattered along the two polypeptide sequences, and no highly conserved regions (i.e., putative recognition motifs for λ) could be identified in any of the two membrane components. It is thus likely that phage λ recognizes a common channel architecture in the inner membrane, rather than specific protein determinants. The phages which do not require the IIC-IID complex for infection either require only the interaction with the outer membrane component to trigger DNA injection or require the participation of another yet unidentified protein or protein complex in the inner membrane to trigger DNA injection.

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